Colony PCR DNA Amplification:

Materials Needed:

PCR Mastermix Supplies
Thermocycler
PCR Tubes
1.5mL Eppendorf Tubes
Pipets
Cell Colonies/ DNA Template

Protocol (estimated time 2-6 hours):

1. Begin by removing all the PCR supplies from the -20°C freezer and thaw them on ice.
2. Fabricate the PCR master max in a 1.5mL eppendorf tube
    a. While making the mix, ensure the tube and its contents are kept on ice.
    b. Add the DNA polymerase (ExTaq) last
    c. Lightly spin the contents of the tube with your wrist prior to adding the extaq polymerase
    d. Depending on how many samples you need to run, refer to table 1 for master mix ingredients.

Table 1: PCR Master Mix							
#PCR Tubes	1	2	3	4	5	6	7
10x Extaq Buffer	2.2	4.4	6.6	8.8	11	13.2	15.4
dH2O	12.65	25.3	37.95	50.6	63.25	75.9	88.55
dNTP	1.76	3.52	5.28	6.93	8.8	10.58	12.32
F Primer	0.44	0.88	1.32	1.76	2.2	2.64	3.08
R Primer	0.44	0.88	1.32	1.76	2.2	2.64	3.08
Polymerase	0.11	0.22	0.33	0.44	0.55	0.66	0.77
Template*	NA	NA	NA	NA	NA	NA	NA

*Template is dependent on the concentration of the thing you want amplified and should not be added to the master mix if performing a colony PCR.

3. Obtain PCR tubes and label them all! (But make sure you remember in which order you place into the PCR machine because the marker does come off occasionally).
4. Pipet 4uL of water into each of the PCR tubes
5. Touch a colony with the pipet tip to get biomass, make sure there is not a lot. Rinse the pipet multiple times in the 4µL of water.
    a. Repeat for any amount of replicates or samples needed

b. Please note, colony PCR can fail therefore we recommend doing at least 2 replicates for any high importance samples.
c. To ensure there was no contamination in the PCR master mix, have a negative control with only master mix
d. To ensure the PCR master mix works, have a positive control from the colony of the bacteria of interest

6. Add 16µL of the master mix into each PCR tube and keep on ice.
7. Take tubes and place them in the thermocycler. Set it up for the correct annealing temperature and elongation time.
    a. Before closing the machine lid, ensure all PCR tubes are closed. If they are open, even slightly, the samples inside will evaporate and become unusable.
    b. See Table 2.

Table 2: PCR Settings (1)		
Step 1	10 minutes at 95°C	Required
Step 2	30 seconds at 94°C	Required
Step 3	30 seconds at 53°C	Variable (primer annealing temperature dependent)
Step 4	1.5 minutes at 72°C	Variable (amplification base pair length dependent)
Step 5	Cycle steps 2 - 4 for 34 times	Variable (dependant on the amount of DNA wanted at end of amplification (2^x))
Step 6	5 minutes at 72°C	Required
Step 7	Infinity at 12°C	Required

8. After all steps are completed and the samples are at 12°C, turn off the thermocycler. Transfer all the tubes into an ice box for use in a PCR clean up, gel electrophoresis, or any other desired application.

References

1. Koch, H. et al. (2013). Diversity and evolutionary patterns of bacterial gut associates of corbiculate bees. *Molecular Ecology, 22(7)*. doi: 10.1111/mec.12209